imply that one need not expect to see marked differences in synthesis of a relatively short runoff transcript by Y639F or the w.t. enzyme when TTP is substituted for UTP.

Lanes u and v show reactions in which two rNTPs have been substituted with dNTPs (dATP and dCTP), and lanes w and x show reactions in which three rNTPs have been substituted with dNTPs (dATP, dCTP, and TTP). Synthesis of the 59 base runoff transcript is observed with the mutant polymerase (lanes v and x) indicating that Y639F can carry out synthesis even when 3 rNTPs are substituted with dNTPs.

While the abortive transcript patterns in lanes v and x may largely be described as a combination of the patterns observed in lanes h and l there are some important distinctions. The transcript patterns in lanes v and x reveal that the structure of the transcript, as well as the structure of the NTP, affect the rate at which NMPs are added to the transcript. For example, in lanes v and x there is an increase in termination at the 6mer length relative to lane l (the 6mer in lanes v or x runs slightly slower than the poly-G 5mer in the adjacent lane). In lanes l, v and x synthesis of the 7mer involves incorporation of dCMP but in lanes v and x the 6mer contains a dAMP at its 3' end and in lane l it contains an rAMP.

The increase in termination at the 6mer point in lanes v or x indicates that the presence of a dNMP on the 3' end of the transcript reduces the ability of the polymerase to further incorporate NMPs. The high level of termination after synthesis of the rGrGrGdA 4mer in lane h relative to the observed termination after synthesis of the rGrGrGrA 4mer in lanes c or d similarly indicates that the presence of deoxynucleotides in the transcript, at least at the 3'-end, influences subsequent extension of the transcript.

Fig. 2 shows the effect of dGTP substitution on transcription by the w.t. and Y639F polymerase. Polymerases, template (supercoiled(Sc) or *Hind*III linearized (Li) pT75), and NTPs were as indicated. Polymerase and template concentrations and electrophoresis conditions as in Fig. 1. *Left panel*: Labeling was with α-P^{32} rGTP (a, d, g, j) or α-P^{32} dGTP(all other lanes). The runoff transcript from the *Hind*III-cut template is indicated in lane f. *Right panel*: Labeling was with α-P^{32}-rGTP (a, b, f, g) or α-P^{32}-dGTP (all other lanes). Poly-rG and poly-dG products of various sizes are indicated in lanes a, c, d. Alignment of these transcript patterns with those in lanes b, c, h, and i in the left panel reveals that the added complexity of the transcript pattern in the latter set of lanes is due to the presence of a mixture of heterogeneous sequence and poly-G transcripts. Heterogenous sequence abortive transcripts are indicated in lane c of the left panel ("4H", "5H").

Fig. 2 reveals the effects of substituting dGTP for rGTP in transcription reactions with the w.t. or Y639F polymerase. In reactions containing only dGTP and *Hind*III-cut pT75 as the template, the mutant polymerase synthesizes poly-dG transcripts up to 4 bases in length (lane c, right panel). Note that--consistent with the assumed structural differences--the poly-dG transcripts ("2dG", "3dG", etc.) in lane c do no co-migrate with the poly-rG transcripts ("2rG", "3rG", etc.) in lane a despite length and sequence identity. When a supercoiled template is used, poly-dG transcripts up to 5 bases in length are obtained (lane d, right panel). In lane e rGMP is added to reactions which contain only dGTP. Ribo-GMP can serve as the initiating, but not elongating nucleotide during transcription(Martin and Coleman, 1989).

With rGMP we therefore ask whether either polymerase can elongate with dGTP if an rNMP is provided for initiation. Addition of rGMP to reactions with dGTP further extends the lengths of the transcripts obtained with the mutant polymerase (lane e, right panel). With the w.t. enzyme very little synthesis is observed in reactions with dGTP (lanes h-j, right panel), though the normal pattern of poly-rG synthesis is observed in the rGTP reactions (lanes f, g, right panel).

When reactions contained three rNTPs and dGTP synthesis of runoff transcript from the *Hind*III-cut template is reduced much more than in reactions in which rGTP is present but other ribonucleotides are substituted with deoxynucleotides. For example, there is very little runoff transcript in lane h of the left panel of Fig. 2. Addition of rGMP increases the amount of runoff transcript made by the mutant enzyme in a reaction containing dGTP (lane i) but the amount of runoff transcript is still much less than in reactions with rGTP. On a supercoiled template (lanes a-c, left panel) high levels of long transcripts are obtained with the mutant enzyme in the reactions with dGTP. The w.t. enzyme shows no transcript synthesis in any of the reactions with dGTP irrespective of whether supercoiled templates or rGMP is used.

Examination of Fig. 2 shows that the marked reduction in runoff transcript synthesis by the mutant enzyme in reactions with dGTP is not due to a deficit in initiation. In fact, in all of the reactions with dGTP we observe abundant synthesis of 2-~6 base transcripts with the mutant enzyme. The low level of runoff transcript synthesis means that these short transcripts are being inefficiently extended to greater lengths. It should also be noted that